Lactobacillus and Lactic Acid Production

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Abstract

Lactobacillus and Production of Lactic Acid. Jessica J. Kious LeTourneau University, Longview, Texas, 75607. National Renewable Energy Laboratory, Golden, Colorado 80401.

Lactic acid is a commercially viable product. The production of lactic acid through fermentation is possible using the microorganism Lactobacillus. A normal strain of Lactobacillus is unable to degrade pentose sugars. However, two strains of Lactobacillus have been discovered--one genetically selected Mont4+ and the other genetically altered, Mont4+pxyAB-mod. Using these strains in an attempt to optimize the production of lactic acid the controls of temperature, sugar type and sugar concentration were tested. Six fermentation experiments were completed with these each of them there was new insight into the conversion of sugar into lactic acid by Lactobacillus. Though all of the controls proved important, temperature and pH seemed to play the largest role in the organisms ability to grow and thus affecting its production of lactic acid. The results are promising for higher yield of lactic acid to be produced using Lactobacillus especially with future investigation into fermentation conducted under pH control.

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Introduction

Lactic acid is a commercially viable product. It is used in such things as: meat and poultry preservation, cosmetics, oral and health care products and baked goods. One way to produced lactic acid is through the fermentation of sugar from the microorganism Lactobacillus. Under optimal conditions of 37^0C and with the sugar glucose present Lactobacillus will convert glucose to lactic acid with one hundred percent yield. However, glucose is only one of many sugars found in nature. More complex pentose sugars such as: L-arabinose (arabinose), D-ribose (ribose) and D-xylose (xylose), are present in vast quantities in herbaceous crops and hardwoods. These materials or biomass are potential sources of energy that the National Renewable Energy Laboratory (NREL) in Golden, Colorado hopes one day soon to be able to turn into an alternative energy source. Yet, Lactobacillus under normal conditions is unable to degrade these complex sugars and without the degradation of these sugars, alternative energy from biomass is limited.

Nevertheless, the Strain development team of the NREL genetically select a strain of Lactobacillus, they call Mont4+, that is able to convert ribose and xylose into lactic acid (Zhang, 1995). The Mont4+ strain is promising in its ability to convert pentose sugars into lactic acid but this strain is still unable to degrade the complex sugar xylose. The Mont4+ was then genetically altered to include a plasmid for the conversion of xylose into lactic acid. The strain that contains this plasmid is known as Mont4+pxyAB-mod (Zhang 1995).

It is from these two strains of Lactobacillus that my research began. Prior to my research, little experimentation had been done outside of fermenting Lactobacillus at

37^0C and with glucose. The Strain Development team wanted to further investigate amounts of lactic acid that would be produced when the sugar types and other conditions were changed using the new strains of Lactobacillus. My research included changing the strain used, temperature, sugar type and sugar concentration. I ran experiments using the L. Mont4+, L. Mont4+pxyAB-mod and an L strain at temperatures of 37^0C, 42^0C and 45^0C. The sugars I used were xylose, arabinose and glucose and the concentrations varied between two to ten percent, depending on the experiment.

Materials and Methods

The first step in my research was to obtain a healthy growing strain of the microorganism. The microorganism was transferred from a frozen "cold" stock solution and incubated with media knows as MRS. The MRS media is specifically suited for Lactobacillus growth and nutrients. The incubation in this initial procedure of strain selection throughout the experiment was 37^0C . The organism was then transferred several times to different test tubes of MRS and the optical density (OD) was checked after every transfer to eliminate possible contaminates and to assure proper growth of the organism. Once the organism was steadily growing it was plated onto MRS plates and allowed to grow for a period of about seventy-two hours. There were several special considerations that had to be taken in this process, one of, which was dependent on the strain of Lactobacillus, and the other applied to all of the strains. The Mont4+pxyAB-mod stain had to be grown, plated and then fermented in the presence of the antibiotic Erythromycin in order to assure that all of the living bacteria contained the plasmid pxyAb-mod. The antibiotic should have killed those without the plasmid. Also, Lactobacillus grows best in anaerobic conditions therefore the plates were placed in a

special gas chamber that locked out all of the air. Therefore, all of the were grown in the gas chamber in the incubator. After the seventy-two hours were complete, with the goal of the plating accomplished individual colonies were isolated apt for pure fermentation.

Once pure colonies had been obtained fermentation could begin. I conducted six separate fermentation experiments. The fermentation was conducted in 200mL bottles with various amounts of MRS, glucose, xylose or arabinose and water. The amount of each of these ingredients depended on the experiment I was conducting. Throughout the experiments the temperature and strains of Lactobacillus were also changed.

My first and second experiments began with only the Mont4+. I incubated at 37^0C and used glucose, varying the concentrations by having two bottles of each of the following: two percent, four percent, six percent, eight percent and ten percent grams per liter. In my third experiment I used glucose solely, incubating at 37^0C, with the Mont4+ and L strain fermenting with two percent, four percent and six percent grams per liter. The fourth and fifth experiments used the same strains, the same concentrations and same sugar as the third, but the temperature was increased to 42^0C in the fourth experiment and 45^0C in the fifth. However, the sixth experiment differed quite a bit from the first five. In the sixth experiment I altered the sugars that I fermented with from just glucose to glucose, arabinose and xylose and I used the plasmid strain of Lactobacillus Mont4+pxyAB-mod. For fermentation, the first bottle was one percent glucose, one percent xylose and one percent arabinose. The second bottle contained no glucose, one percent xylose and one percent arabinose. The third bottle had no glucose and no arabinose, it contained only one percent xylose. The fourth bottle had only one percent arabinose. The fifth bottle had no arabinose instead it was one percent xylose and one

percent glucose. The sixth bottle had one percent glucose and one percent arabinose. All of experiment six was fermented at 37^0C.

This is a basic layout of how my experiments were conducted. In the following paragraphs I will explain in further detail the sampling procedures and tests that were conducted with each experiment.

For every bottle in every experiment three-milliliter samples were taken twice daily. The samples were taken in the morning and in the evening. I took approximately 330 samples throughout the course of the experiment. It was important to take the samples under sterile conditions to insure no contamination occurred that might affect the growth of the organism and give incorrect data in further sampling. While sampling was being conducted the bottles were removed from the incubator. After sampling was complete the bottles were immediately placed back in the incubator to continue growing. Though this step may seem trivial to include it is important to mention for the integrity of the research to show that the bottles were not just left out after taking samples.

The three-milliliter samples, mentioned above, were critical to completing the three tests that I conducted throughout my research, taken on every sample from every bottle. These tests were: pH monitoring, HPLC and OD. The pH monitoring was important to the experiment for several reasons, one of which is that Lactobacillus is an organism that is able to grow at a low pH—approximately 3.5 – 4 (please see pictograph for picture). This low pH allows for the screening out of possible contaminants. Also, when Lactobacillus is grown at a higher pH or a pH that is near neutral, it produces a lactic salt. This lactic salt is in an unusable form and the process of converting it into the functional form of lactic acid is expensive and produces unwanted waste. Therefore, the

pH is my experiment was monitored but it was not controlled. The pH monitoring was conducted using a pH meter. Each pH reading was recorded in data tables and analyzed at the end of the experiment. Along with the pH being able to keep out other possible bacteria, the pH was a good indicator of whether or not sugar was being converted into lactic acid. For example, as the pH began to drop since lactic acid is more acid, giving the lower pH, I knew the sugar was being used.

The testing done for the HPLC was more time consuming than that of the pH monitoring. HPLC is an acronym for High Pressure Liquid Chromatography. This is a machine that uses a special column to run purified liquid samples and to determine how much xylose, arabinose, glucose, acetic acid, lactic acid, ethanol and glycerol are in each sample, (for a picture please refer to pictographs). Since the HPLC is very sensitive to contaminants all of the cells from each sample must be filtered out using special syringes and filters. The water used to dilute each sample must also be sterile filtered through vacuum filtration. For my experiment, I used a dilution of five times the sample to water in one-milliliter viles. Each vile was sealed with a special instrument and specifically labeled to run through the HPLC.

Testing for the optical density (OD) of the microorganism was done with the use of the spectrophotometer (please see the picture in the pictographs). The spectrophotometer uses light fractionation to analyze how much light passes through a container and from the amount of light passing though it can calculate how many particles there are in that container. I used a one milliliter cuvette throughout my experiment for the container. For the zero time point of each experiment since the growth was low there was no dilution done before taking the OD. For the first time point

only a two-fold dilution was done and from that point throughout the rest of the experiment a ten-fold dilution was used for OD measurements. The OD test was the best way to obtain immediate results of whether or not the organism was growing. As the organism grew more of them were present in the sample and thus the OD increased.

These three tests and the sampling mentioned above are the basic components that make up the methods and materials of my experiment. It is the data generated from these tests that gave me the information needed to generate the results I will discuss in the following paragraphs.

Results

The easiest way to break down my results is to go through the data experiment by experiment-- beginning with the second experiment. For the second experiment the data I generated, (please see the attached data tables) showed that with the Mont4+ strain, as the concentration of glucose increased there was not a significant increase in the amount of lactic acid produced. However, the increase in glucose did produce a slight increase in the amount of lactic acid produced, even though it was small. A startling thing I found in this experiment was that the OD was very low. The maximum OD for Lactobacillus to be a growing strain should be between six and eight. The highest OD I achieved in this experiment was 4.49.

The controls in the third experiment were not terribly different from the second. The only differences were that the L strain was run simultaneously with the Mont4+ and the concentration of glucose only went from two percent to six percent gram per liter vs. the ten percent grams per liter in the second experiment. In the third experiment, there is an increase in lactic acid with the increase in glucose. Yet, again the increase is very

small and it varied from organism to organism. The Mont4+ had the highest yield of lactic acid fermenting with six percent concentration of glucose, whereas the L strain utilized the sugar best at the four percent concentration. There was also an increase in the OD in this experiment, as compared to the second experiment; the OD in the third experiment did reach up to the normal growing rate of between 6.57 to 7.51.

The fourth experiment, incubating at the higher temperature of 42^0C, did not yield as much lactic acid as the third experiment. The highest amount of lactic acid in the fourth experiment for Mont4+ was 17.17 g/L at the six percent concentration of glucose as compared to 28.309 g/L at the same concentration. The same was also true for the L strain—less Lactic acid was produced at 42^0C using the same concentration of glucose than in was at 32^0C in experiment three. The L strain in experiment four produced a maximum amount of 20.9 g/L lactic acid in the fourth experiment at six percent concentration and 23.9 in the third experiment. The OD was also significantly lower in the forth experiment as compared to the third. Another interesting dimension I added to this experiment was that I ran two stains of Mont4+ simultaneously. I found that there were differences between these two strains,(that should be identical), with their pH, OD and the amount of lactic acid produced.

The fifth experiment used the same controls as the third and fourth with only temperature being increased from 42^0C to 45^0C. At this higher temperature I found similar trends as were noted above in the forth experiment--the OD decreased again and the lactic acid produced was less. The yield of total lactic acid was the lowest at 45^0C.

The sixth experiment was really in a league all of its own. Not only was the organism changed from Mont4+ and L strain, to the plasmid strain Mont4+pxyAB-mod,

but I also changed the sugar from just glucose to glucose, xylose and arabinose. I found glucose was consumed first in all of the bottles that contained glucose. However, in those bottles that did not contain glucose, xylose and arabinose were consumed simultaneously. Also, once arabinose was consumed though both arabinose and xylose were consumed simultaneously, arabinose was consumed faster than xylose and after arabinose was consumed the consumption of xylose continued. I also discovered that when the pH would drop to about four the consumption of sugar would significantly decrease. Nonetheless, the plasmid stain did ferment all of the sugars and this was a very important factor to note. For example, in the bottle, that only arabinose was present, all of the arabinose was converted to lactic acid. The same is true of xylose. Both xylose and arabinose when fermented at a concentration of one percent with the Mont4+pxyAB-mod produced approximately ten grams per liter of lactic acid.

Discussion and Conclusion

The results of my experiments are quite promising. I was able to complete six full rounds of fermentation and from each of these I gained a great deal of information that I believe will contribute to the knowledge of the Strain Development team. By changing the controls of temperature, organism, sugar type and sugar concentration I was able to draw some new conclusions that before this experimentation were unknown about Lactobacillus.

I found the best condition for lactic acid production during fermentation is 37^0C and a higher concentration of glucose such as four or six percent grams per liter. However, the effect of this increase in sugar concentration is not dramatic (Figures 1 and 2). I also found that increasing the temperature with the Mont4+ and L strains of

Lactobacillus caused a decrease in the growth, or optical density, of the organism. The increase in temperature also caused a decrease in the utilization of glucose. I believe the limiting factor in both of these cases was the pH. I found that as the pH would approach four there was little glucose consumption and therefore no lactic acid produced. It is possible that the higher temperature brought too much stress on the organisms metabolic abilities.

Regarding the pH, as the pH drops it appears to limit the growth of the organism. This point was clearly shown in the sixth experiment. In bottle one where xylose, arabinose and glucose were all present in one percent the glucose consumption happened very quickly—within five hours. After the glucose is consumed abainose is degraded and by the time a portion of arabinose is broken down the pH is already at 3.56. Xylose can be degraded at a lower pH and I believe this is part of the reason it is 'saved' until the end. However, once the pH of the media reaches below four the organism can no longer grow and use the sugars. Other evidence of pH seriously affecting OD and sugar utilization is found in the fact that at the when fermenting at the higher temperatures there is a great deal of unused glucose. For example, in experiment five with the Mont4+ at six percent concentration of glucose, by the end of the run there is still 44.7grams of glucose remaining in the sample out of the original 60.7grams. The pH at this point is 4.1. I believe if the pH was maintained at a higher number, such as six, the organism would have a possibility to continue growing and use the full amount of sugar.

It is possible that my stain of Mont4+ was contaminated. In the forth experiment when I ran the two strains of Mont4+ and got different results for pH, OD and lactic acid production these are good indications that one of the strains was contaminated. If this

experiment were conducted again it would be crucial that the Mont4+ be genetically examined to insure that it is indeed the stain originally screened for its ability to ferment pentose sugars.

Though I learned and generated a great deal of information regarding Lactobacillus, there is definitely room for more research to be done. Particularly research using the plasmid strain of Mont4+ as well as Mont4+ itself. Future research may include controlling the pH during fermentation and genetically screening the Mon4+ strain to affirm that the strain used is not contaminated. Lactobacillus is a very promising microorganism and with the right type of research conducted NREL may be one step closer to offering the world an new alternative energy source.

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I would like to thank the United States Department of Energy- Office of Science for giving me the opportunity to work in such a start-of-the-art facility and for funding the Energy Research Undergraduate Laboratory Fellowship Program.

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FIGURES

Experiment #2 Varying Amounts of Glucose on Mon 4+ Fermenting at 37C

2% Glucose **HPLC**

1A

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.53	0.081	20.198	0.796	1.83	0	0
6/27 3:50pm	T1	4.5	6.41	0.172	20.28861	0.963	1.81	0	0
6/28 9:30am	T2	22	3.88	3	12.28538	8.030	1.912	0	0
6/28 6:00pm	T3	30.5	3.76	3.28	10.19586	9.545	1.86	0	0
6/29 9:00am	T4	45.5	3.49	3.85	7.91546	12.110	1.92	0	0
6/29 3:15pm	T5	51.5	3.45	3.83	7.12169	12.50	1.91	0	0
6/30 10:30am	T6	71	3.34	4.24	5.05852	13.30	4.83	0	0
6/30 3:15pm	T7	75.5	3.34	4.78	4.46822	13.64	1.77	0	0
7/3 11:30am	T8	143.5	3.37	4.81	1.56809	17.809	2.02	0	0

2% Glucose **HPLC**

1B

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.61	0.083	19.45684	0.754	1.75	0	0
6/27 3:50pm	T1	4.5	6.4	0.156	19.04268	1.007	1.85	0	0
6/28 9:30am	T2	22	3.87	2.93	12.47196	7.812	1.92	0	0
6/28 6:00pm	T3	30.5	3.77	3.25	11.07434	9.477	1.90	0	0
6/29 9:00am	T4	45.5	3.49	3.73	8.2484	11.66	1.92	0	0
6/29 3:15pm	T5	51.5	3.46	4.14	7.99024	12.78	2.02	0	0
6/30 10:30am	T6	71	3.32	3.94	6.16995	14.55	2.03	0	0
6/30 3:15pm	T7	75.5	3.32	4.35	5.19746	13.98	1.91	0	0
7/3 11:30am	T8	143.5	3.37	4.56	1.93334	17.38	2.00	0	0

4% Glucose **HPLC**

2A

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.58	0.079	45.399	0.864	1.95	0	0
6/27 3:50pm	T1	4.5	6.34	0.162	43.0440	1.025	1.84	0	0
6/28 9:30am	T2	22	3.86	2.83	17.9844	21.021	3.878	0	0
6/28 6:00pm	T3	30.5	3.76	3.17	30.5664	9.095	1.84	0	0
6/29 9:00am	T4	45.5	3.49	3.54	29.12157	11.64	1.92	0	0
6/29 3:15pm	T5	51.5	3.46	3.74	28.5242	12.25	1.93	0	0
6/30 10:30am	T6	71	3.34	3.97	26.5927	13.61	1.93	0	0
6/30 3:15pm	T7	75.5	3.32	4.26	25.2979	13.94	1.901	0	0
7/3 11:30am	T8	143.5	3.38	4.5	23.4634	16.97	1.95	0	0

4% Glucose **HPLC**

2B

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.59	0.072	41.49536	0.787	1.82	0	0
6/27 3:50pm	T1	4.5	6.38	0.15	39.8263	1.0166	1.89	0	0
6/28 9:30am	T2	22	3.86	2.91	32.39117	7.613	1.85	0	0
6/28 6:00pm	T3	30.5	3.76	3.28	37.6670	11.491	2.310	0	0
6/29 9:00am	T4	45.5	3.49	3.74	28.6840	11.83	1.921	0	0
6/29 3:15pm	T5	51.5	3.46	3.93	27.2687	12.06	1.89	0	0
6/30 10:30am	T6	71	3.33	3.96	26.5444	14.01	1.95	0	0
6/30 3:15pm	T7	75.5	3.32	2.96	25.7616	13.77	1.85	0	0
7/3 11:30am	T8	143.5	3.38	4.49	23.51404	17.25	1.99	0	0

6% Glucose **HPLC**

3A

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.55	0.075	63.7735	0.805	1.82	0	0
6/27 3:50pm	T1	4.5	6.32	0.15	57.2524	1.012	1.85	0	0
6/28 9:30am	T2	22	3.85	2.81	38.7667	21.156	3.89	0	0
6/28 6:00pm	T3	30.5	3.76	3.21	51.12548	9.241	1.88	0	0
6/29 9:00am	T4	45.5	3.5	2.66	48.71662	11.23	1.89	0	0
6/29 3:15pm	T5	51.5	3.46	3.68	43.18128	10.77	1.73	0	0
6/30 10:30am	T6	71	3.35	3.74	48.2026	13.92	1.99	0	0
6/30 3:15pm	T7	75.5	3.32	3.91	45.0399	13.94	1.83	0	0
7/3 11:30am	T8	143.5	3.38	4.52	44.83961	16.68	1.99	0	0

6% Glucose **HPLC**

3B

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.55	0.073	62.16456	0.718	1.75	0	0
6/27 3:50pm	T1	4.5	6.31	0.152	59.34701	1.034	1.85	0	0
6/28 9:30am	T2	22	3.83	3.01	52.39071	7.636	1.86	0	0
6/28 6:00pm	T3	30.5	3.74	3.23	52.19696	9.873	1.93	0	0
6/29 9:00am	T4	45.5	3.45	3.59	48.46146	11.82	1.88	0	0
6/29 3:15pm	T5	51.5	3.44	3.75	47.2882	12.33	1.91	0	0
6/30 10:30am	T6	71	3.31	4.02	49.12347	14.90	2.02	0	0
6/30 3:15pm	T7	75.5	3.3	4.1	38.7256	11.63	1.58	0	0
7/3 11:30am	T8	143.5	3.39	4.44	44.4077	17.17	1.95	0	0

8% Glucose **HPLC**

4A

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.52	0.07	81.16225	0.773	1.70	0	0
6/27 3:50pm	T1	4.5	6.35	0.12	79.58213	0.996	1.90	0	0
6/28 9:30am	T2	22	3.85	2.71	58.64012	21.08	3.79	0	0
6/28 6:00pm	T3	30.5	3.75	3.02	71.29631	9.125	1.85	0	0
6/29 9:00am	T4	45.5	3.48	3.43	64.00361	10.25	1.74	0	0
6/29 3:15pm	T5	51.5	3.45	3.56	65.36118	11.121	1.79	0	0
6/30 10:30am	T6	71	3.34	3.61	68.5866	13.55	1.92	0	0
6/30 3:15pm	T7	75.5	3.32	4.09	63.17657	13.28	1.80	0	0
7/3 11:30am	T8	143.5	3.38	4.24	66.4047	16.32	1.94	0	0

8% Glucose **HPLC**

4B

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.52	0.069	83.2444	0.796	1.76	0	0
6/27 3:50pm	T1	4.5	6.32	0.132	71.93942	0.914	1.69	0	0
6/28 9:30am	T2	22	3.84	2.75	73.5634	7.628	1.856	0	0
6/28 6:00pm	T3	30.5	3.75	3.07	72.3627	9.575	1.87	0	0
6/29 9:00am	T4	45.5	3.48	4.03	68.9908	11.529	1.87	0	0
6/29 3:15pm	T5	51.5	3.45	3.7	59.0032	10.40	1.70	0	0
6/30 10:30am	T6	71	3.34	3.88	65.6663	13.39	1.92	0	0
6/30 3:15pm	T7	75.5	3.31	3.8	61.26267	9.577	1.34	0	0
7/3 11:30am	T8	143.5	3.39	4.34	64.82381	16.49	1.98	0	0

10% Glucose **HPLC**

5A

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.49	0.07	101.82	0.781	1.76	0	0
6/27 3:50pm	T1	4.5	6.3	0.118	104.339	1.042	1.95	0	0
6/28 9:30am	T2	22	3.84	2.63					
6/28 6:00pm	T3	30.5	3.75	3.09	94.5384	9.519	1.93	0	0
6/29 9:00am	T4	45.5	3.49	3.28	89.58193	11.30	1.89	0	0
6/29 3:15pm	T5	51.5	3.47	3.11	85.77915	11.36	1.84	0	0
6/30 10:30am	T6	71	3.34	3.19	89.0505	13.68	1.95	0	0
6/30 3:15pm	T7	75.5	3.32	3.71					
7/3 11:30am	T8	143.5		4.09	86.4656	16.39	2.00	0	0

10% Glucose **HPLC**

5B

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	LA	AA	Gly	Eth
6/27 11:15am	T0	0	6.5	0.067	101.2370	0.776	1.74	0	0
6/27 3:50pm	T1	4.5	6.29	0.128	97.7857	0.995	1.90	0	0
6/28 9:30am	T2	22	3.83	2.55	94.23291	7.746	1.90	0	0
6/28 6:00pm	T3	30.5	3.75	3.07	91.90957	9.394	1.88	0	0
6/29 9:00am	T4	45.5	3.49	3.3	87.6899	11.17	1.899	0	0
6/29 3:15pm	T5	51.5	3.45	3.42	86.61327	11.72	1.88	0	0
6/30 10:30am	T6	71	3.33	3.57	86.6692	13.56	1.96	0	0
6/30 3:15pm	T7	75.5	3.32	2.98	86.17597	13.79	1.84	0	0
7/3 11:30am	T8	143.5	3.38	4.08	85.0436	16.41	2.00	0	0

Experiment #3

Varying Amounts of Glucose on Mon 4+ Fermenting at 37C

2% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
T0 7/11 11:45am	T0	0	6.44	0.227	20.0966	1.90829	3.80265	0	0
T1 7/11 4:45pm	T1	6	6.07	0.516	20.16284	2.72506	3.87782	0	0
T2 7/12 11:10am	T2	24.5	4.11	4.47	3.00139	18.04736	3.84761	0	0
T3 7/12 5:05pm	T3	30.5	3.96	5	6.33369	15.05462	3.80595	0	0
T4 7/13 10:20am	T4	42.5	3.75	6.49	2.53612	18.6599	3.8894	0	0
T5 7/13 4:25pm	T5	54.5	3.69	6.28	1.4055	18.81645	3.76213	0	0
T6 7/14 6:00pm	T6	80.5	3.72	6.6	0	21.61073	3.95467	0	0

4% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
T0 7/11 11:45am	T0	0	6.42	0.22	41.40729	1.95634	3.8584	0	0
T1 7/11 4:45pm	T1	6	6.07	0.474	40.41557	2.5624	3.8106	0	0
T2 7/12 11:10am	T2	24.5	4.13	4.48	30.31745	12.93266	3.85415	0	0
T3 7/12 5:05pm	T3	30.5	3.96	4.88	27.4370	14.82191	3.82359	0	0
T4 7/13 10:20am	T4	42.5	3.73	5.86	23.5262	18.66679	3.88606	0	0
T5 7/13 4:25pm	T5	54.5	3.69	5.85	21.73514	19.22985	3.82458	0	0
T6 7/14 6:00pm	T6	80.5	3.65	6.61	19.02565	22.74144	3.94772	0	0

6% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
T0 7/11 11:45am	T0	0	6.42	0.22	62.38021	1.96304	3.94311	0	0
T1 7/11 4:45pm	T1	6	6.07	0.478	60.0247	2.69616	3.76116	0	0
T2 7/12 11:10am	T2	24.5	4.03	4.8	48.0025	13.7693	3.84249	0	0
T3 7/12 5:05pm	T3	30.5	3.88	5.13	47.0822	16.10148	3.78661	0	0
T4 7/13 10:20am	T4	42.5	3.67	6.39	42.11824	19.60276	3.78239	0	0
T5 7/13 4:25pm	T5	54.5	3.64	6.29	40.9370	20.46954	3.79192	0	0
T6 7/14 6:00pm	T6	80.5	3.65	6.81	12.70357	28.30911	3.9255	0	0

2% Glucose **HPLC**

L.

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
T0 7/11 11:45am	T0	0	6.46	0.223	20.20981	1.94165	3.83801	0	0
T1 7/11 4:45pm	T1	6	6.11	0.51	20.0656	2.76887	3.97858	0	0
T2 7/12 11:10am	T2	24.5	3.81	6.23	8.70128	13.12794	3.89632	0	0
T3 7/12 5:05pm	T3	30.5	3.7	6.51	0.55006	20.09754	3.82236	0	0
T4 7/13 10:20am	T4	42.5	3.65	6.5	0	20.94104	3.827	0	0
T5 7/13 4:25pm	T5	54.5	3.64	6.88	0	20.58507	3.77804	0	0
T6 7/14 6:00pm	T6	80.5	3.76	6.57	0	21.40533	3.90795	0	0

4% Glucose **HPLC**

L.

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
T0 7/11 11:45am	T0	0	6.44	0.231	40.6638	1.92622	3.85026	0	0
T1 7/11 4:45pm	T1	6	6.11	0.478	62.0748	2.95821	3.89777	0	0
T2 7/12 11:10am	T2	24.5	3.79	6.3	23.7674	17.96791	3.8162	0	0
T3 7/12 5:05pm	T3	30.5	3.68	6.75	21.63591	20.29379	3.85379	0	0
T4 7/13 10:20am	T4	42.5	3.54	7.21	17.01225	24.09339	3.85604	0	0
T5 7/13 4:25pm	T5	54.5	3.51	7.12	16.21496	25.15998	3.8732	0	0
T6 7/14 6:00pm	T6	80.5	3.54.	7.51	32.8727	28.75079	3.9529	0	0

6% Glucose **HPLC**

L.

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
T0 7/11 11:45am	T0	0	6.42	0.207	55.8993	1.75671	3.51503	0	0
T1 7/11 4:45pm	T1	6	6.05	0.48	40.5274	2.72998	3.82723	0	0
T2 7/12 11:10am	T2	24.5	3.8	6.33	44.0650	17.80679	3.83202	0	0
T3 7/12 5:05pm	T3	30.5	3.68	6.67	42.71254	20.10472	3.79181	0	0
T4 7/13 10:20am	T4	42.5	3.52	7.49	37.88481	24.19252	3.80246	0	0
T5 7/13 4:25pm	T5	54.5	3.5	7.04	37.0314	25.28266	3.83748	0	0
T6 7/14 6:00pm	T6	80.5	3.52	7.41	38.417	23.87786	3.8931	0	0

Experiment #4

Varying Amounts of Glucose on L. Fermenting at 42C

2% Glucose **HPLC**

L.

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.5	0.078	19.79906	0.674508	1.66666	0	0
8/1 4:35pm	T1	2	5.47	0.436	19.17182	1.53998	1.67853	0	0
8/2 10:35am	T2	18	3.95	2.72	10.8126	8.45201	1.60356	0	0
8/2 5:00pm	T3	26.5	4.01	3.05	10.09224	9.68103	1.66758	0	0
8/3 11:35am	T4	43	3.74	3.35	8.22464	11.76318	1.71217	0	0
8/3 4:55pm	T5	50.5	3.86	3.56	7.59484	11.48381	1.65643	0	0
8/4 10:00am	T6	65.5	3.65	2.9	6.61434	12.52322	1.64435	0	0
8/4 5:30pm	T7	75	4	2.69	6.52623	13.02414	1.69661	0	0
8/7 11:30am	T8	141	3.6	3.19	5.25442	14.18122	1.7028	0	0
8/7 5:00pm	T9	146.5	3.59	3.76	5.17079	14.1571	1.71142	0	0

4% Glucose **HPLC**

L.

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.32	0.008	40.11907	1.49392	3.48375	0	0
8/1 4:35pm	T1	2	5.69	0.532	39.2747	2.44726	3.50883	0	0
8/2 10:35am	T2	18	4.11	5.23	26.4379	13.1288	3.35972	0	0
8/2 5:00pm	T3	26.5	4.18	4.26	25.2436	14.17779	3.40854	0	0
8/3 11:35am	T4	43	3.94	4.87	24.176	16.82802	3.54521	0	0
8/3 4:55pm	T5	50.5	4.06	4.98	23.25104	16.68586	3.48757	0	0
8/4 10:00am	T6	65.5	3.85	4.06	20.09912	16.20335	3.16668	0	0
8/4 5:30pm	T7	75	4.2	3.93	12.72914	10.47815	1.98198	0	0
8/7 11:30am	T8	141	3.79	4.69	20.4925	19.64891	3.47994	0	0
8/7 5:00pm	T9	146.5	3.79	6.28	20.3333	19.75854	3.5463	0	0

6% Glucose **HPLC**

L.

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.3	0.006	60.8845	1.49657	3.54498	0	0
8/1 4:35pm	T1	2	5.7	0.462	58.9247	2.431124	3.50023	0	0
8/2 10:35am	T2	18	4.05	5.51	45.2658	14.2751	3.48907	0	0
8/2 5:00pm	T3	26.5	4.11	4.37	44.87361	15.44621	3.461	0	0
8/3 11:35am	T4	43	3.88	4.76	43.0297	17.64704	3.56782	0	0
8/3 4:55pm	T5	50.5	4	5.05	42.12908	17.61439	3.51447	0	0
8/4 10:00am	T6	65.5	3.79	4.1	28.3407	13.02857	2.39943	0	0
8/4 5:30pm	T7	75	4.16	3.93	40.69187	18.88951	3.53299	0	0
8/7 11:30am	T8	141	3.77	4.62	39.21453	20.1045	3.4623	0	0
8/7 5:00pm	T9	146.5	3.75	5.5	39.5608	20.40094	3.4937	0	0

Experiment #4

Varying Amounts of Glucose with Mon 4+ Fermenting at 42C

2% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
7/18 2:30pm	T0	0	6.38	0.021	20.20652	1.44044	3.55987	0	0
7/18 5:00pm	T1	2.5		0.124	19.04548	1.81769	3.43554	0	0
7/19 10:20am	T2	13	4.3	3.72	10.13265	10.89786	3.57077	0	0
7/19 4:25pm	T3	23.5	4.16	4.2	8.94159	12.77788	3.55288	0	0
7/20 11:00am	T4	37.5	4	4.9	5.43718	15.02205	3.56806	0	0
7/20 5:00pm	T5	48	3.97	4.86	5.09557	16.45407	3.71221	0	0
7/21 11:00am	T6	61.5	3.89	2.66	3.2615	16.95906	3.59709	0	0
7/21 4:35pm	T7	71.5	3.86	2.48	3.05	17.10275	3.5435	0	0

4% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
7/18 2:30pm	T0	0	6.37	0.02	38.72847	1.33662	3.38687	0	0
7/18 5:00pm	T1	2.5	6.17	0.09	40.7846	1.72909	3.46077	0	0
7/19 10:20am	T2	13	4.31	3.63	31.44235	10.69591	3.53758	0	0
7/19 4:25pm	T3	23.5	4.17	3.22	29.51165	12.24515	3.44447	0	0
7/20 11:00am	T4	37.5	4	4.72	26.59201	14.69666	3.52974	0	0
7/20 5:00pm	T5	48	3.98	4.63	25.70813	15.13643	3.51113	0	0
7/21 11:00am	T6	61.5	3.9	2.33	24.95713	16.85078	3.64035	0	0
7/21 4:35pm	T7	71.5	3.88	2.47	23.85089	16.48223	3.49376	0	0

6% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
7/18 2:30pm	T0	0	6.35	0.02	57.95061	1.32175	3.34271	0	0
7/18 5:00pm	T1	2.5	6.03	0.122	53.25268	1.71177	3.03802	0	0
7/19 10:20am	T2	13	4.26	3.8	50.97568	11.22123	3.55788	0	0
7/19 4:25pm	T3	23.5	4.13	4.02	48.00292	12.74183	3.49021	0	0
7/20 11:00am	T4	37.5	3.97	4.78	46.23527	15.39104	3.49085	0	0
7/20 5:00pm	T5	48	3.94	4.58	44.41639	15.5397	3.39856	0	0
7/21 11:00am	T6	61.5	3.87	2.31	44.04872	17.25204	3.52534	0	0
7/21 4:35pm	T7	71.5	3.84	2.65	43.02433	17.17064	3.46433	0	0

Experiment #5

Varying Amounts of Glucose in Mon 4+ & L. Fermenting at 45C

2% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.35	0.021	20.11639	1.57223	3.5476	0	0
8/1 4:35pm	T1	2	5.81	0.496	15.86521	1.92705	2.88262	0	0
8/2 10:35am	T2	18	4.3	5.21	9.50687	11.096	3.46073	0	0
8/2 5:00pm	T3	26.5	4.32	3.97	8.28085	11.71347	3.46639	0	0
8/3 11:35am	T4	43	4.11	4.38	6.97481	13.93779	3.63234	0	0
8/3 4:55pm	T5	50.5	4.19	5.34	6.52103	13.6954	3.54355	0	0
8/4 10:00am	T6	65.5	4.01	3.79	4.88323	12.92476	3.11967	0	0
8/4 5:30pm	T7	75	4.35	3.64	5.41676	14.90361	3.57058	0	0
8/7 11:30am	T8	141	3.96	4.33	4.12596	16.23362	3.58766	0	0
8/7 5:00pm	T9	146.5	3.95	5.33	4.08614	16.18921	3.59606	0	0

4% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.29	0.015	40.27752	1.55127	3.48858	0	0
8/1 4:35pm	T1	2	5.82	0.474	39.3082	2.29885	3.44405	0	0
8/2 10:35am	T2	18	4.26	3.35	27.5250	10.52702	3.17612	0	0
8/2 5:00pm	T3	26.5	4.29	4.14	26.21998	11.24844	3.21469	0	0
8/3 11:35am	T4	43	4.07	4.43	27.30671	14.46423	3.60732	0	0
8/3 4:55pm	T5	50.5	4.16	5.85	27.65441	14.99928	3.72712	0	0
8/4 10:00am	T6	65.5	3.97	3.96	25.31233	15.20276	3.53374	0	0
8/4 5:30pm	T7	75	4.32	4.01	25.31026	15.50544	3.56915	0	0
8/7 11:30am	T8	141	3.93	4.22	14.99992	10.49191	2.21039	0	0
8/7 5:00pm	T9	146.5	3.91	4.52	23.71689	16.60507	3.54875	0	0

6% Glucose **HPLC**

Mon4+

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.31	0.014	60.6906	1.5303	3.48819	0	0
8/1 4:35pm	T1	2	5.79	0.422	59.8288	2.28966	3.44691	0	0
8/2 10:35am	T2	18	4.24	3.38	50.51784	11.55091	3.46203	0	0
8/2 5:00pm	T3	26.5	4.28	3.99	48.09681	11.84167	3.42763	0	0
8/3 11:35am	T4	43	4.07	4.32	48.32944	14.32622	3.53445	0	0
8/3 4:55pm	T5	50.5	4.14	6.4	46.8434	14.19323	3.44576	0	0
8/4 10:00am	T6	65.5	3.97	3.69	40.17822	13.09547	3.04139	0	0
8/4 5:30pm	T7	75	4.31	3.52	45.3600	15.30646	3.44048	0	0
8/7 11:30am	T8	141	3.92	4.1	44.51722	16.6797	3.46728	0	0
8/7 5:00pm	T9	146.5	3.91	5.72	44.7458	16.79846	3.49322	0	0

Experiment #5

Varying amounts of Glucose in L. & Mon4+ fermenting at 45C

2% Glucose L. **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.47	0.042	20.09014	0.83472	2.08432	0	0
8/1 4:35pm	T1	2	5.77	0.382	19.18106	1.35989	2.03104	0	0
8/2 10:35am	T2	18	3.9	3.92	8.85984	11.09591	2.09013	0	0
8/2 5:00pm	T3	26.5	3.98	3.35	7.58422	11.49077	2.00811	0	0
8/3 11:35am	T4	43	3.75	3.54	5.91994	14.05356	2.12732	0	0
8/3 4:55pm	T5	50.5	3.88	3.77	5.22211	13.04245	1.85021	0	0
8/4 10:00am	T6	65.5	3.66	3.02	5.22062	15.379	2.22157	0	0
8/4 5:30pm	T7	75	4.03	3.03	4.89685	14.82301	2.11782	0	0
8/7 11:30am	T8	141	3.66	3.49	4.35861	15.39857	2.16388	0	0
8/7 5:00pm	T9	146.5	3.64	3.57	3.9282	14.00731	1.91094	0	0

4% Glucose L. **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.32	0.007	40.6657	1.15228	3.57237	0	0
8/1 4:35pm	T1	2	5.87	0.402	39.6708	2.10656	3.45628	0	0
8/2 10:35am	T2	18	4.08	4.83	26.9439	13.84265	3.42424	0	0
8/2 5:00pm	T3	26.5	4.17	1.66	24.72103	13.82229	3.25516	0	0
8/3 11:35am	T4	43	3.96	4.28	24.46531	16.50763	3.56237	0	0
8/3 4:55pm	T5	50.5	4.08	4.65	27.9446	18.91778	3.97931	0	0
8/4 10:00am	T6	65.5	3.9	3.85	23.37901	17.22304	3.53613	0	0
8/4 5:30pm	T7	75	4.23	3.62	23.15556	17.2416	3.50317	0	0
8/7 11:30am	T8	141	3.87	4.17	22.6070	18.28439	3.59232	0	0
8/7 5:00pm	T9	146.5	3.85	4.79	22.4907	17.96112	3.53165	0	0

6% Glucose L. **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Lactic Acid	Acetic Acid	Gly	Eth
8/1 2:45pm	T0	0	6.32	0.006	61.65921	1.52859	3.53262	0	0
8/1 4:35pm	T1	2	5.9	0.376	55.7068	1.9229	3.2864	0	0
8/2 10:35am	T2	18	4	5.01	45.18134	14.82473	3.42401	0	0
8/2 5:00pm	T3	26.5	4.08	3.75	43.5886	15.76815	3.46224	0	0
8/3 11:35am	T4	43	3.87	4.43	42.7329	18.07832	3.57343	0	0
8/3 4:55pm	T5	50.5	3.98	4.53	47.0159	20.19526	3.84723	0	0
8/4 10:00am	T6	65.5	3.81	3.71	41.80015	19.28304	3.60582	0	0
8/4 5:30pm	T7	75	4.16	3.77	41.22852	19.13225	3.50041	0	0
8/7 11:30am	T8	141	3.78	4.13	40.91673	20.0346	3.52518	0	0
8/7 5:00pm	T9	146.5	3.77	4.41	40.41835	19.84822	3.48777	0	0

Experiment #6

Varying sugars Ara., Xyl., and Glucose in Mon4+pxyAB-mod

#1 **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Xylose	Arabin.	Lactic Acid	Acetic Acid	Gly	Eth
8/2 10:30am	T0	0	6.62	0.163	9.28871	8.88341	9.421	0.557116	1.58789	0	1.58074
8/2 5:00pm	T1	5.5	6.48	0.08	9.36589	9.08839	9.71437	0.662629	1.62331	0	1.62051
8/3 11:30am	T2	25	3.78	4.13	0	8.62564	9.60948	10.15885	1.64191	0	1.62171
8/3 4:55pm	T3	29.5	3.87	4.15	0	8.44844	9.60155	10.90105	1.64502	0	1.62949
8/4 10:00am	T4	47.5	3.65	2.52	0	7.09238	9.40351	12.50925	1.71859	0	1.59733
8/4 5:30pm	T5	54	4.01	2.25	0	6.82726	9.23221	12.72595	1.72833	0	1.60194
8/7 11:30am	T6	120	3.57	3.11	0	6.34896	8.37005	14.2268	1.72198	0	1.62336
8/7 5:00pm	T7	125.5	3.56	3.18	0	6.38179	8.41841	14.24451	1.73769	0	1.60757

#2 **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Xylose	Arabin.	Lactic Acid	Acetic Acid	Gly	Eth
8/2 10:30am	T0	0	6.63	0.162	0	8.76859	9.42975	0.550659	1.57617	0	1.53282
8/2 5:00pm	T1	5.5	6.52	0.07	0	9.15362	9.8497	0.673694	1.64869	0	1.55443
8/3 11:30am	T2	25	4.03	2.85	0	8.23626	3.28493	7.57953	1.63982	0	1.5734
8/3 4:55pm	T3	29.5	4	2.96	0	7.87934	1.8712	8.81779	1.59465	0	1.57147
8/4 10:00am	T4	47.5	3.6	2.95	0	5.58255	0	13.53709	1.68498	0	1.56722
8/4 5:30pm	T5	54	3.96	2.89	0	5.00196	0	14.1974	1.65123	0	1.56703
8/7 11:30am	T6	120	3.52	2.57	0	2.96359	0	16.14503	1.65971	0	1.55899
8/7 5:00pm	T7	125.5	3.5	3.18	0	2.9014	0	16.01999	1.65791	0	1.55338

#3 **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Xylose	Arabin.	Lactic Acid	Acetic Acid	Gly	Eth
8/2 10:30am	T0	0	6.66	0.164	0	8.90687	0	0.567579	1.61079	0	1.51169
8/2 5:00pm	T1	5.5	6.55	0.07	0	8.90632	0	0.646034	1.6159	0	1.52924
8/3 11:30am	T2	25	6	0.29	0	8.74795	0	1.2541	1.69396	0	1.57252
8/3 4:55pm	T3	29.5	5.86	0.33	0	8.33069	0	1.3221	1.62183	0	1.52053
8/4 10:00am	T4	47.5	4.57	1.11	0	5.16268	0	4.33326	1.64669	0	1.53368
8/4 5:30pm	T5	54	4.64	1.5	0	3.80877	0	5.74675	1.62553	0	1.52748
8/7 11:30am	T6	120	3.89	1.68	0	0	0	9.39045	1.63372	0	1.52365
8/7 5:00pm	T7	125.5	3.88	1.63	0	0	0	9.44363	1.63822	0	1.54142

#4 **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Xylose	Arabin.	Lactic Acid	Acetic Acid	Gly	Eth
8/2 10:30am	T0	0	6.66	0.162	0	0	9.51546	0.56979	1.60302	0	1.547
8/2 5:00pm	T1	5.5	6.55	0.07	0	0	9.68795	0.651652	1.64501	0	158204
8/3 11:30am	T2	25	4.11	2.66	0	0	3.01552	7.09044	1.65471	0	1.59304
8/3 4:55pm	T3	29.5	4.03	3.12	0	0	1.06289	8.66902	1.86947	0	2.08112
8/4 10:00am	T4	47.5	3.82	1.29	0	0	0	10.01288	1.61033	0	1.55253
8/4 5:30pm	T5	54	4.18	1.21	0	0	0	10.06629	1.69296	0	1.58321
8/7 11:30am	T6	120	3.83	2.03	0	0	0	9.9595	1.60851	0	1.57545
8/7 5:00pm	T7	125.5	3.83	2.77	0	0	0	10.01082	1.66798	0	1.57809

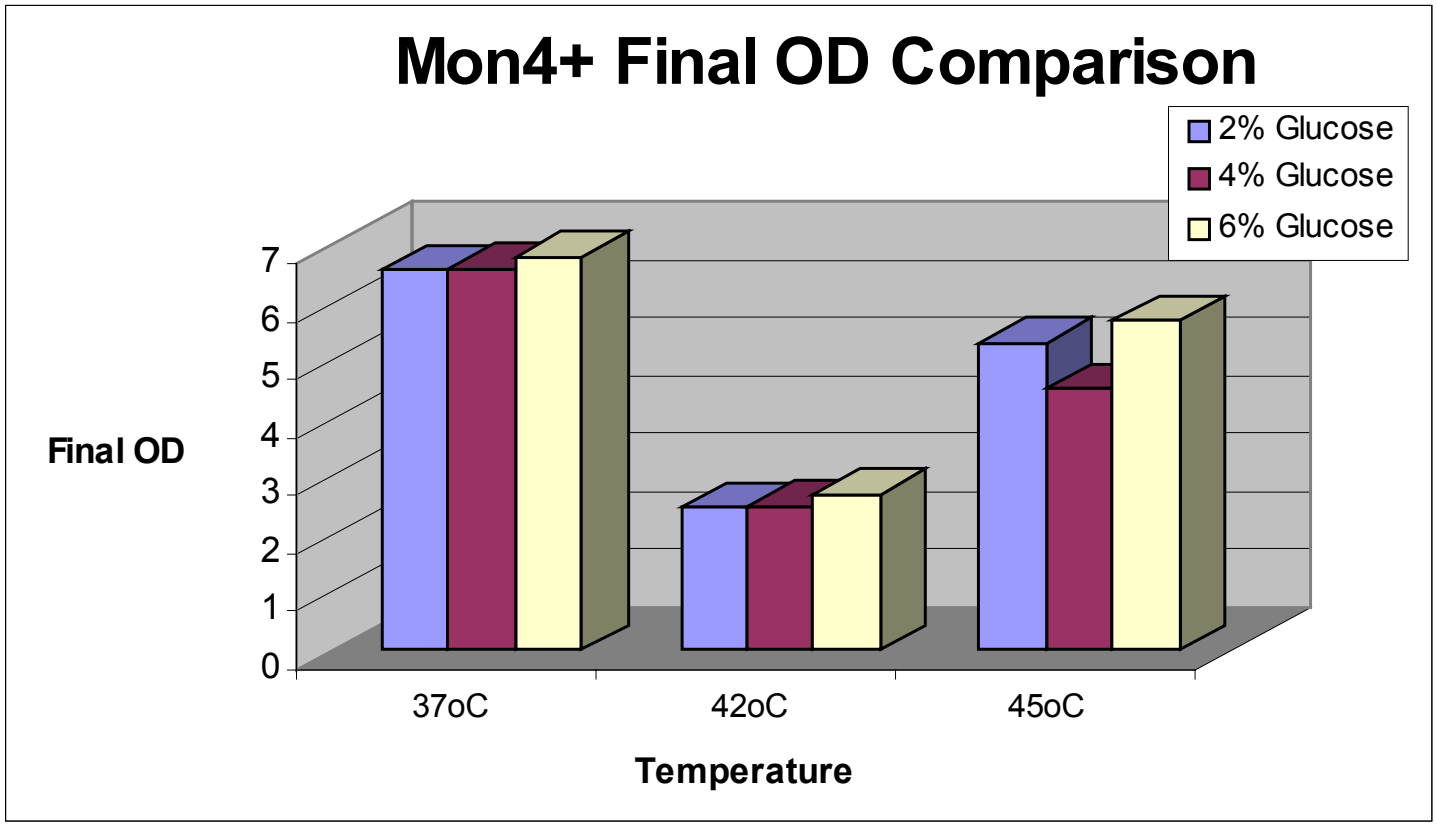
#5 **HPLC**

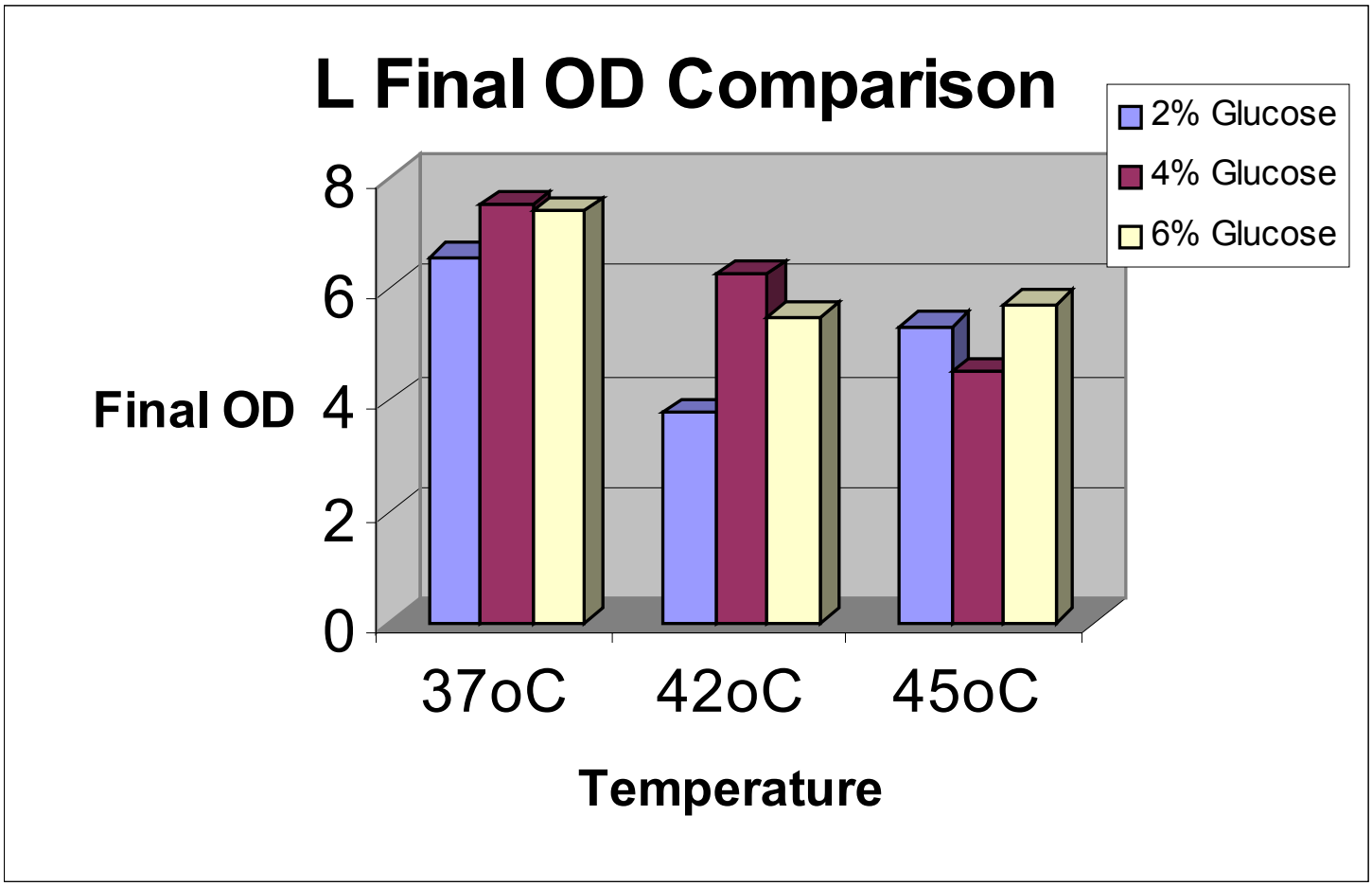
Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Xylose	Arabin.	Lactic Acid	Acetic Acid	Gly	Eth
8/2 10:30am	T0	0	6.66	0.163	9.13484	8.8029	0	0.554858	1.56776	0	1.54906
8/2 5:00pm	T1	5.5	6.58	0.09	9.72098	9.4468	0	0.689397	1.68232	0	1.6449
8/3 11:30am	T2	25	3.81	4.19	0	8.66758	0	10.18849	1.60253	0	1.61942
8/3 4:55pm	T3	29.5	3.92	4.19	0	8.15875	0	10.89254	1.85366	0	2.12732
8/4 10:00am	T4	47.5	3.66	2.42	0	7.07017	0	12.01568	1.66825	0	1.61224
8/4 5:30pm	T5	54	4.03	2.11	0	6.89457	0	12.11364	1.65157	0	1.60024
8/7 11:30am	T6	120	3.65	2.79	0	6.39947	0	12.88159	1.68103	0	1.61484
8/7 5:00pm	T7	125.5	3.64	2.77	0	6.23473	0	12.51254	1.66479	0	1.61002

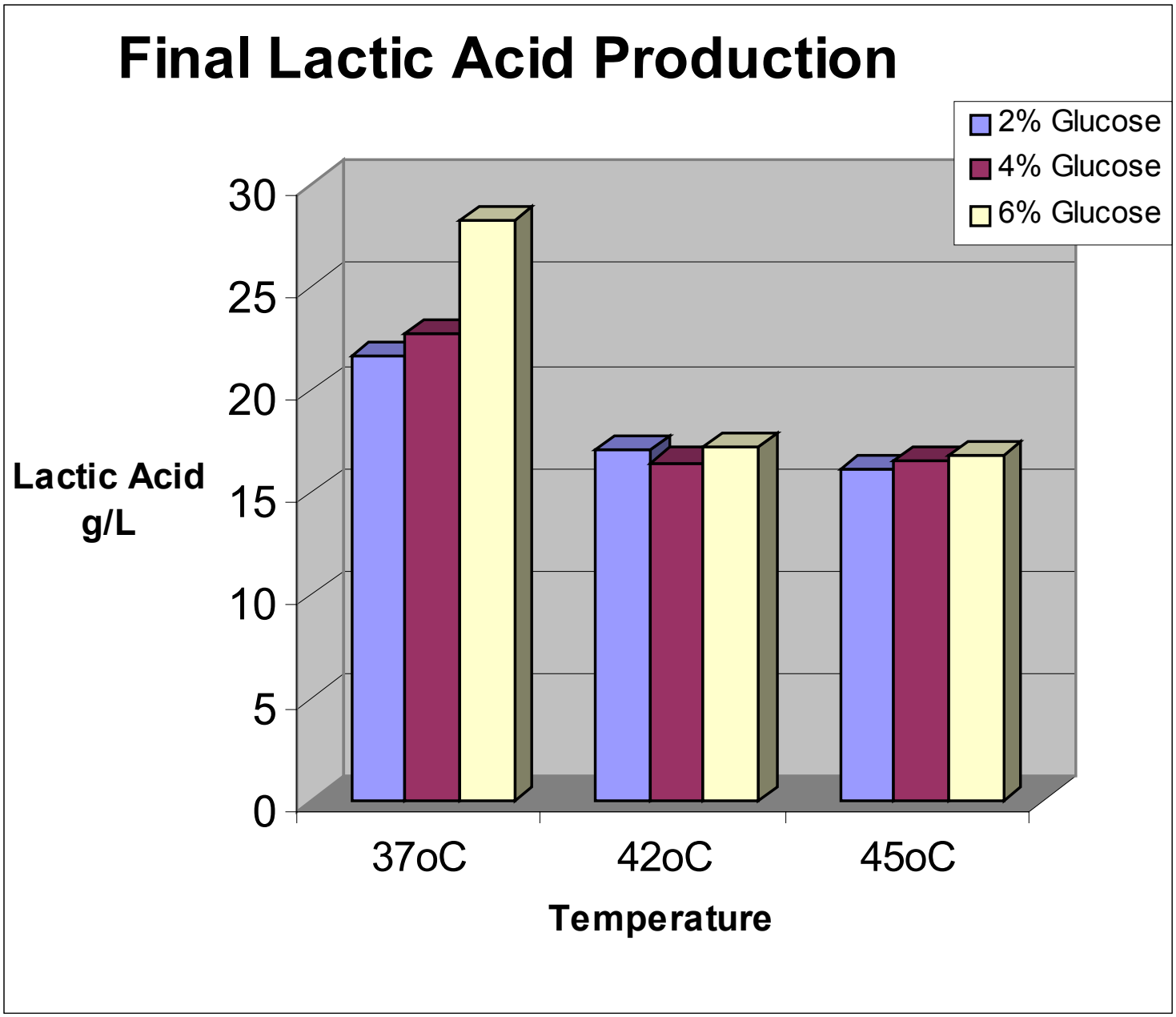
#6 **HPLC**

Date and Time	Time Pt.	Hrs.	PH	OD	Glucose	Xylose	Arabin.	Lactic Acid	Acetic Acid	Gly	Eth
8/2 10:30am	T0	0	6.66	0.16	8.57369	0	8.64456	0.464951	1.43015	0	1.48082
8/2 5:00pm	T1	5.5	6.57	0.11	9.08114	0	9.17346	0.599277	1.53323	0	1.56555
8/3 11:30am	T2	25	3.82	4	0	0	9.69141	10.01624	1.60133	0	1.65897
8/3 4:55pm	T3	29.5	3.92	3.9	0	0	9.41851	10.22656	1.51164	0	1.62213
8/4 10:00am	T4	47.5	3.77	1.8	0	0	9.5281	10.4569	1.56825	0	1.65368
8/4 5:30pm	T5	54	4.14	1.71	0	0	9.55252	10.48995	1.57334	0	1.65391
8/7 11:30am	T6	120	3.77	3.02	0	0	9.20019	10.69122	1.60753	0	1.62018
8/7 5:00pm	T7	125.5	3.76	3.08	0	0	9.36299	10.81844	1.58267	0	1.64281

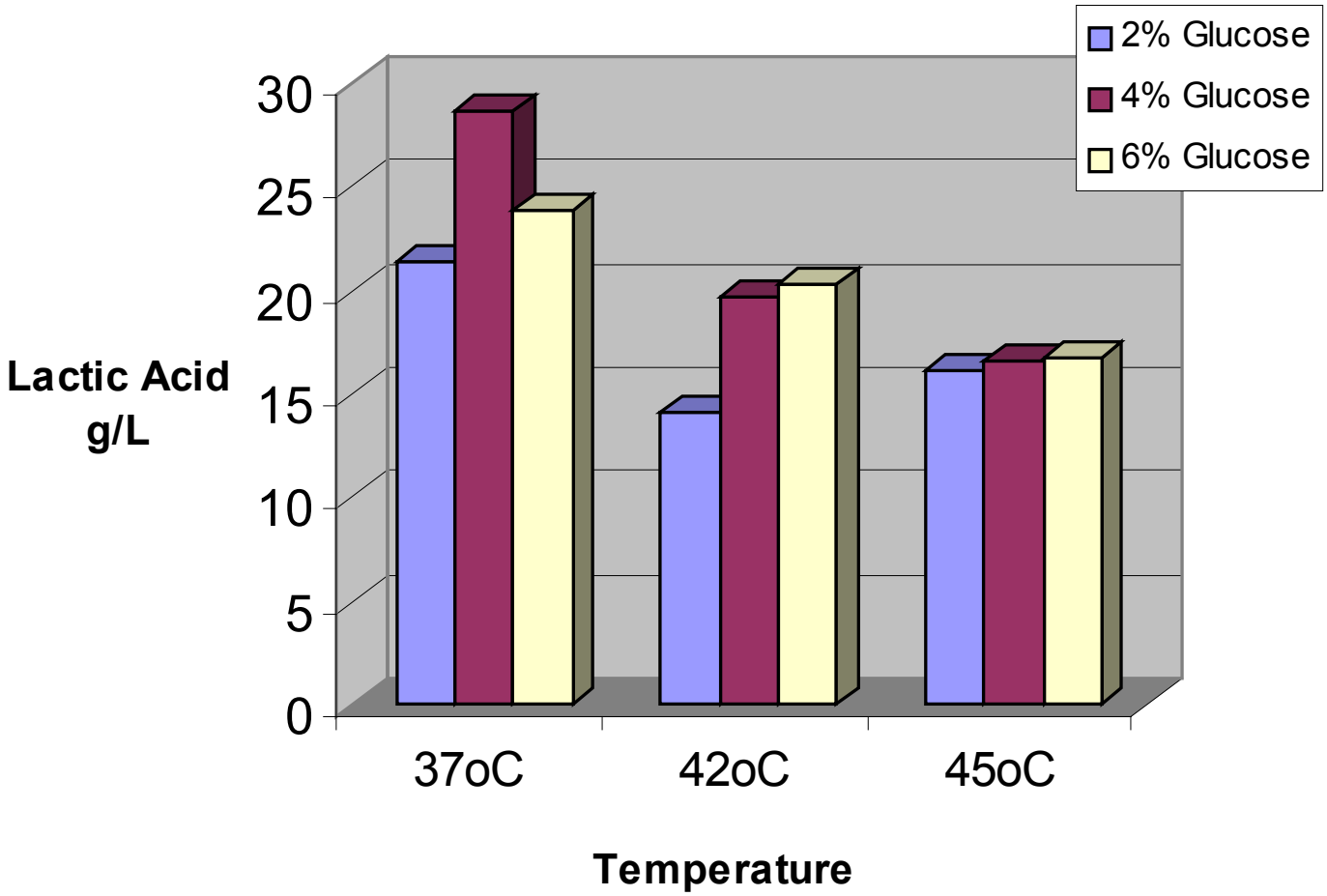
PHOTOMICROGRAPHS

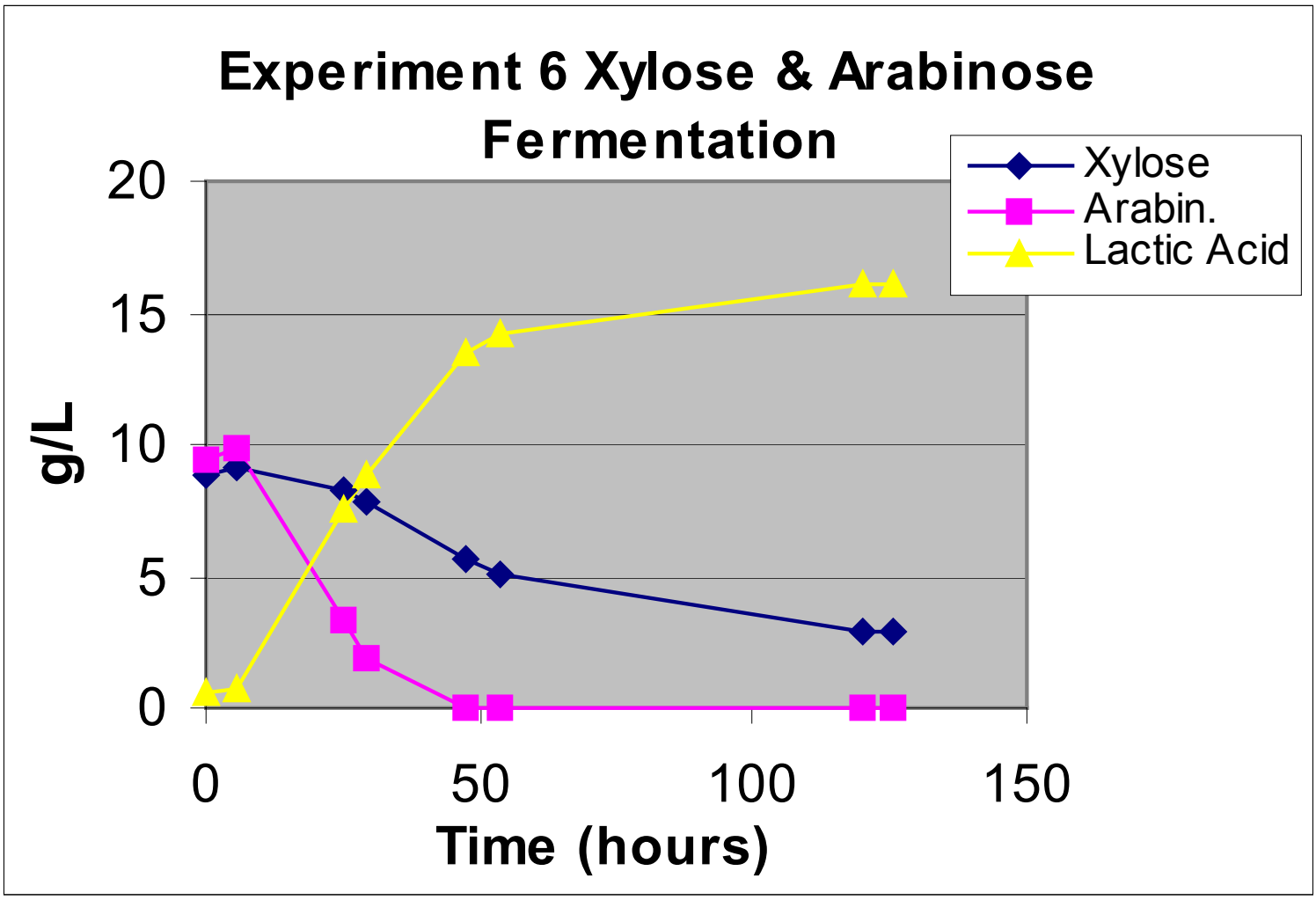


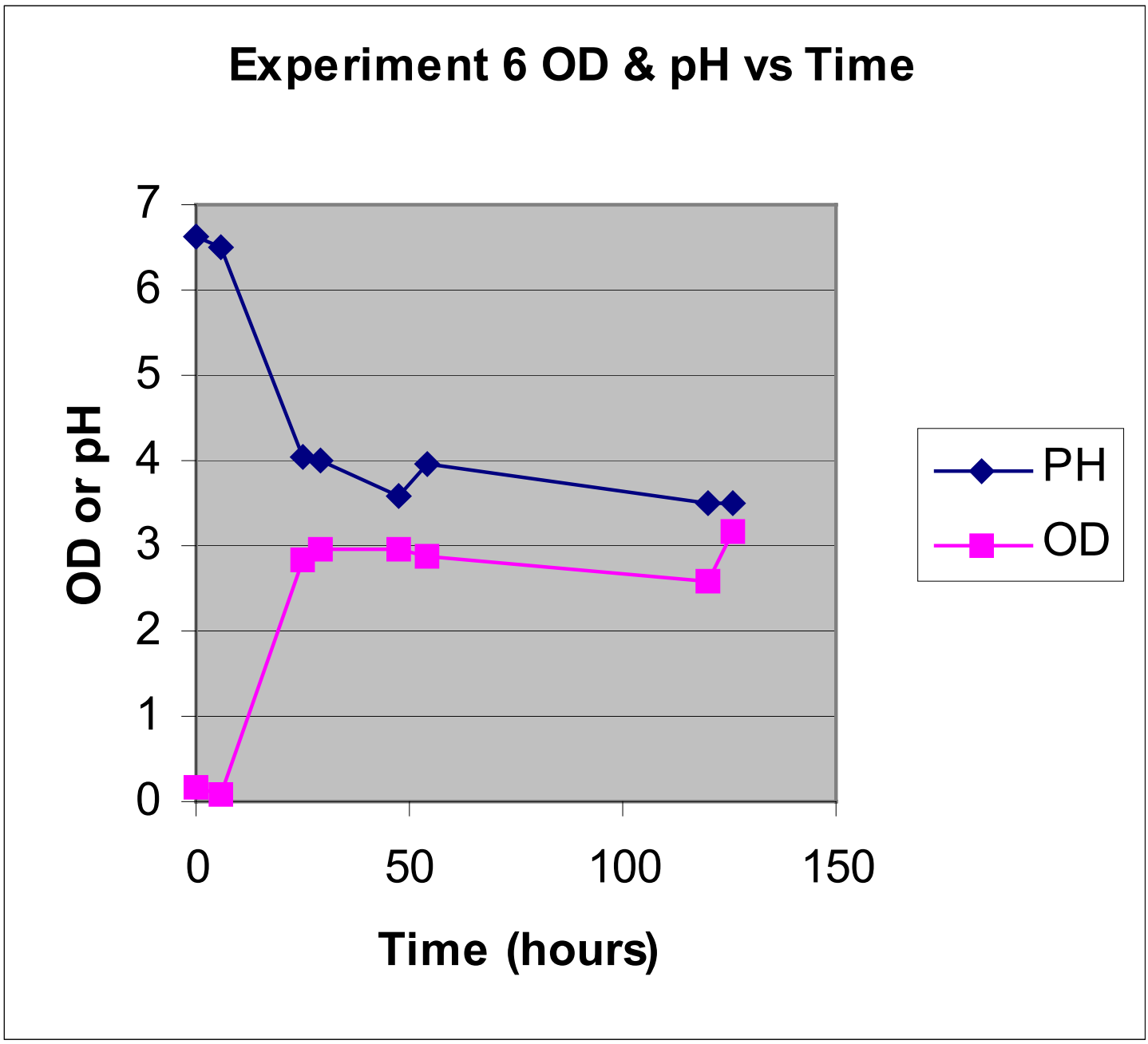

L Final OD Comparison
2% Glucose
4% Glucose
6% Glucose
Final OD
8
6
4
2
0
37oC
42oC
45oC
Temperature


Final Lactic Acid Production
2% Glucose
4% Glucose
6% Glucose
30
25
20
15
10
5
0
Lactic Acid g/L
37oC
42oC
45oC
Temperature

Final Lactic Acid Producation




Experiment 6 Xylose & Arabinose Fermentation
Xylose
Arabin.
Lactic Acid
20
15
10
5
0
g/L
0
50
100
150
Time (hours)


Experiment 6 OD & pH vs Time
OD or pH
7
6
5
4
3
2
1
0
0
50
100
150
Time (hours)
PH
OD